

Zelle 4/4/50

H226 Single cell isolations

3. 3/31/50

	Lac	Mal	EMS	<u>Lac</u>
A . 8	+	+	+	
10	+	+	+	
19	+	+	+	
23	-	-	o	
24	-	-	o	
25	+	+	+	
26	+	+	+	
27	+	++	+	
28	+	+	+	
31	+	+	+	
32	+	+	+	
33	+	+	+	
34	+	++	+	
41	+	++	+	
42	+	+	+	
59	+	+	+	
60	+	+	+	
61	+	+	+	
62	+	+	+	
10	+	+	+	
17	+	+	+	
27	+	+	+	
20	+	+	+	
25	+	+	+	
27	+	++	+	
28	+	+	+	
29	+	+	+	
37	+	+	+	
38	+	+	+	
39	+	+	+	
40	+	+	+	
53	+	++	+	
54	+	+	+	
61	-	+	-	
62	+	++	+	

A23-24

A25-26

B61

B62 B29

F21 are segregants. F22  
 These sibs are indicated —.

Transfer sibs to D(Lac); also streak out  
 on several sugars.

! Check for partial segregation!

O = n.g.  
 + = var.g.

Var Mal EMS Var

17

18

19

20

21

22

23

24

25

26

27

28

31

32

33

34

59

60

61

62

all<sup>x</sup>all<sup>x</sup>all<sup>x</sup>

12

17

18

19

20

21

22

23

24

28

30

31

32

33

34

56

111

112

119

120

121

122

all<sup>x</sup>all<sup>x</sup>all<sup>x</sup>

2	+	+
4	+	+
16	+	+
17	+	+

+	+
+	+
+	+
+	+

C

D

E

Irac Ural Sac

10

16

17

18

23

24

25

26

27

29

31

all x

32

57

58

61

62

83

84

85

86

19

all x

all x

12

16

17

18

19

20

21

22

23

24

27

28

29

31

32

61

62

all x

all x

288 screw caps returned to Zelle.



K 15 < 32°  
 K 16 < 33°  
 3 < 8 < 18°  
 9 < 19°  
 K 4 < 10°  
 10 < 21°  
 11 < 23°  
 12 < 25°  
 13 < 27°  
 14 < 29° 60°  
 15 < 30° 61°  
 16 < 31°  
 3 < 15°  
 5 < 18°  
 7 < 19°  
 9 < 20°  
 K 4 < 10°  
 10 < 21°  
 11 < 24°  
 12 < 26°  
 13 < 28° 57°  
 14 < 30° 62°

Probably most of these are  
 haploid cells. They grow too  
 well. Inverted microscope work  
 on 18 hour Davis minimal  
 culture. (Inoculated with an  
 EMAS live colony)

I can put the batch in the vials OK, have them while  
 waiting for bugs to grow. Won't have another chance  
 till next week - tracing again.

Var Mal ENS Lac



9	+	+	+
17	+	+	+
21	-	+	0
22	+	+	+
24x	0	0	0
25x	0	0	0
27x	0	0	0
31	++	++	++
32	++	++	++
33	+	++	++
34	++	++	++
37	++	++	++
47x	0	0	0
48x	0	0	0
53x	0	0	0
57x	0	0	0
58x	0	0	0

check!

17  
18  
19  
20  
21  
22

23

24

25

26

27

28

31

32

33

34

59

60

61

62

allx

allx

allx

Study of Zelle single cell isolations

726 b.

A. segregants: Lac Mal Xyl Mtl Nutr.

1	A23	-	-	-	-	T <sup>L</sup> B <sub>1</sub>
2	A24	-	-	-	-	T <sup>L</sup> B <sub>1</sub>
3	B61	-	+	+	+	H <sup>T</sup> B <sub>1</sub>
4	F21	-	+	+	+	H <sup>T</sup> B <sub>1</sub> , repeat: ✓ no partial segregants.

B sib's

1	A25	✓	✓	✓	✓	+
2	A26	✓	✓	✓	✓	+
3	B29	✓	✓	✓	✓	+
4	B62	✓	✓	✓	✓	+
5	F22	✓	✓	✓	✓	+

} no deviations

C doubtful v.

F 31	✓	✓ <sup>+</sup>
32	✓	✓ <sup>+</sup>
33	✓	✓ <sup>+</sup>
34	0	0
37	✓	✓ <sup>+</sup>
B 27	✓	✓ <sup>+</sup>
34	✓	✓ <sup>+</sup>
41	✓	✓ <sup>+</sup>

} no deviations.

April 7, 1950.

These are cultures described on p. 700.

A. P7 Strains on 698B1, B2 700-2. p8. allow that.

1 698 B1 #2 is Lac+, with some for most part.

2 B2

3 700-2 = 698B2! #1, 3 are apparently mixtures of Lac+, Lac-.

Replies apparent pure +.

- ① 6 colonies streaked out. Each throws off ca 1% Lac-! Note u.
- ② 4 colonies " ". Mostly +, some v?, a few % -.
- ③ " " ". 1% -; mostly type +.

Test ~~Xyl~~, Lac- is on Xyl, Mtl; Nutrition of parent.

Check nutrition of single + colonies from ①, ③

Also inoculate in O(N<sub>2</sub>ase) lac for mutation.

1. 8 Lac- : Mtl+ Xyl+
2. 6 Lac- : all Mtl+; 5 Xyl-; 1 Xyl+.
3. 6 Lac- : Mtl+ Xyl+.

See 731

April 9, 1950.

Show ~~727-1~~ and ~~727-3~~ in ~~D-Y2~~ - Phl. aer. est. 10<sup>10</sup> yield.  
 Dilute each 10<sup>-4</sup> for irradiation. 20 sec 30 cm.  
 Dilute to 10<sup>-6</sup>. controls .05  
 4 v .1 of this dilution/plate.

1. Control.	"+"	"v"	s	-
	117	13	1	31
	107	10	0	25

1 UV=x	227	40	46

Negligible killing. Repeat expt. with higher doses and better populations

Thal + survivors of 727:1,3

~~727-1~~ Pick papillae from EMB Mal and purify. No frank Thal<sub>v</sub>.  
 Pick to lac EMB; streak out on EMB Mal.

$$\begin{matrix} MA = \\ MB = \end{matrix} \frac{727_1}{727_3}$$

A: 10 picked and purified 4 Mal+ from EMB Mal brushed on EMB lac #3 and 6 were lac- #10a, b lac- c, d lac+

B. All 7(4) lac+. streaks out on Thal EMB for hemi-zymotic test.

A: all lac ++, - Thal pure ++.

∴ 727-1 and 727-3

B: all lac ++, - Thal pure ++.

are pure Thal -

Hemizygosity tests: ~~H237~~ H237

728

4/9/50.

see 720. From W67 x W950. Lac v Xyl, Mal-Gal- MH? St?  
check from slant. Dose. 0(Lac) 10 ml for heavy suspensions.

H237 may not be heterozygous diploid: it does not  
give typical lac<sub>v</sub>.

One Mal+	3 Gal+	each pure +.
----------	--------	--------------

but lac appearance peculiar.  
Recheck H237. appears to  
be Lac +.

April 9, 1950.

20 secs. 50 cm. Irradiate 14226 1/3 at  $10^{-4}$  ml H<sub>2</sub>O.

Dilute ~~to~~ to  $\frac{1}{10^6}$ ; plate .1 ml

A Control

B UV.

EMB Lac

A	32	15
	55	22
	28	15
B	2	12
	6	7
	6	16
	2	15

Not a highly pure suspension.

EMS Lac — A - too many lac -

G.G.

# Fermentation of nolactose

730

April 8, 1950

- |   |        |           |           |
|---|--------|-----------|-----------|
| A | 58-161 | 1/100 ml  | Y2 1% lac |
| B | "      | 3 x 25 ml | " agar    |
| C | W1301  | 1/100 ml  | "         |
| D | "      | " agar    | "         |

	Flesh	Cells	substr.
1	2A	B	-
2	9B	B	glucose 10mg.
3	3A	B	lactose "
4	4B	B	nolac "
5	6A	D	-
6	10A	D	glucose 10mg
7	11A	D	lactose "
8	14A	D	nolac "
T			

This experiment was designed to determine whether lactose-adapted cells could utilize nolactose.

Since 58-161, fully lactose-adapted, ferments nolactose  $\frac{1}{4}$  as rapidly as it does lactose, the non-fermentability may be due to a block of adaptation comparable to lac: Bugal or lac: - . However, the falling off of the fermentation may speak for inhibition by lactose, since a relatively high K<sub>s</sub> for nolactose!

	T	1	2	3	4	5	6	7	8
12 <sup>20</sup>	137 <sup>+39</sup>	22	17 <sup>+</sup>	-2	25+	50	30	20	7
12 <sup>25</sup>	86 <sup>9</sup>	23	17 <sup>+</sup>	-1	25	50	26-38 (-30)	21	8

12 <sup>30</sup>	140	27	95	34	30+	58+ <sup>85</sup> <del>85</del>	54	11
12 <sup>35</sup>	140	26	163	90	34	55	145	106
12 <sup>40</sup>	141	27	269	186	51	58	244 283 to 68	203
			<sup>795 to</sup> <sup>(+211) 84</sup>	<sup>217 to</sup> <sup>69148</sup>			<sup>242 to</sup> <sup>75</sup> [167]	41
12 <sup>45</sup>	143	30	155	124- <sup>114</sup>	77	60	136	129
12 <sup>50</sup>	145	33	251	213 <sup>312</sup>	105	62	230	214
12 <sup>55</sup>	142	33	165	<sup>+488</sup> <sup>273-96</sup>	98 <sup>329</sup>	63	<sup>361-53</sup> <sup>+338</sup>	117
12 <sup>60</sup>	143	34	249	172	159	64	210	208
12 <sup>65</sup>	144	34	181	214	208	66	<sup>251-79</sup> <sup>+510, 27</sup>	204
				379			<sup>306, 71</sup>	261
								265-112

	T	1	2	3	4	5	6	7	8
1 <u>45</u>	148	35	250	274	229	64	258	147	141
		+ 272-110	+ 803	297-88 <del>588</del>	247-123 <del>(125)</del>			269-82	
1 <u>35</u>	150	40	184	170	154	66	164	228	181
		+ 931	232-114	219-126 <del>681</del>				263-118	
1 <u>35</u>	149	40	150	162	198	20	249	172	234
							269-73		247-404 296
1 <u>45</u>	150	43	205	219	230	70	118	231	140.
1 <u>55</u>	154	46	246	262	261	75	161	275	180
		+ 254-110	267-119 <del>629</del>	273-116 <del>282</del>				285-113	
2 <u>05</u>	154	43	129	131	134	71	199	136	215
2 <u>15</u>	152	45	147	151	163	74	204	163	241
2 <u>25</u>	150	47	159	161	187	76	217	168	270
									278-115
2 <u>35</u>	150	43	162	163	201	77	230	165	130
2 <u>45</u>	154	48	172	166	221	77	235	165	148

4/14/50. Cells had been kept in refrigerator  
 Estimate optical density at  $4200\text{\AA}$ . dilute in  
 distilled water

A  $10^{-2}$  287

B  $10^{-2}$  281

min.	T	Th	1	2	3	4	5	6	7	8
0	1230	0	0							
5	1235	0	-1	68	56	4	-1	60	52	5
10	40	-1	-1	173	151	20	-1	158	148	29
15	45	-3	0	268	235	44	1	263	239	63
20	50	-5	1	362	322	70	1	355	322	101
25	55	-2	4	456	401	99	5	446	398	141
30	150	-3	4	539	474	126	5	545	499	190
40	10	-4	3	623	565	174	6	633	594	246
45	15	-8	0	688	621	191	0	760	668	275
55	25	-10	3	782	724	239	0	851	747	313
65	35	-9	4	867	810	284	6	937	837	367
75	45	-12	4	919	864	313	2	999	893	413
85	55	-14	5	958	905	342	1	1040	935	451
95	205	-14	2	985	922	372	1	1078	968	486
105	15	-12	6	1005	944	364	6	1085	997	514
115	22	-12	8	1117	954	427	8	1098		543
125	35	-10	6	1022	958	446	11	1113		568
135	245	-14	7	1028	957	459	7	1114		582

Flask const. 1.84 1.64 1.69 1.81 1.58 1.60 1.70 1.58

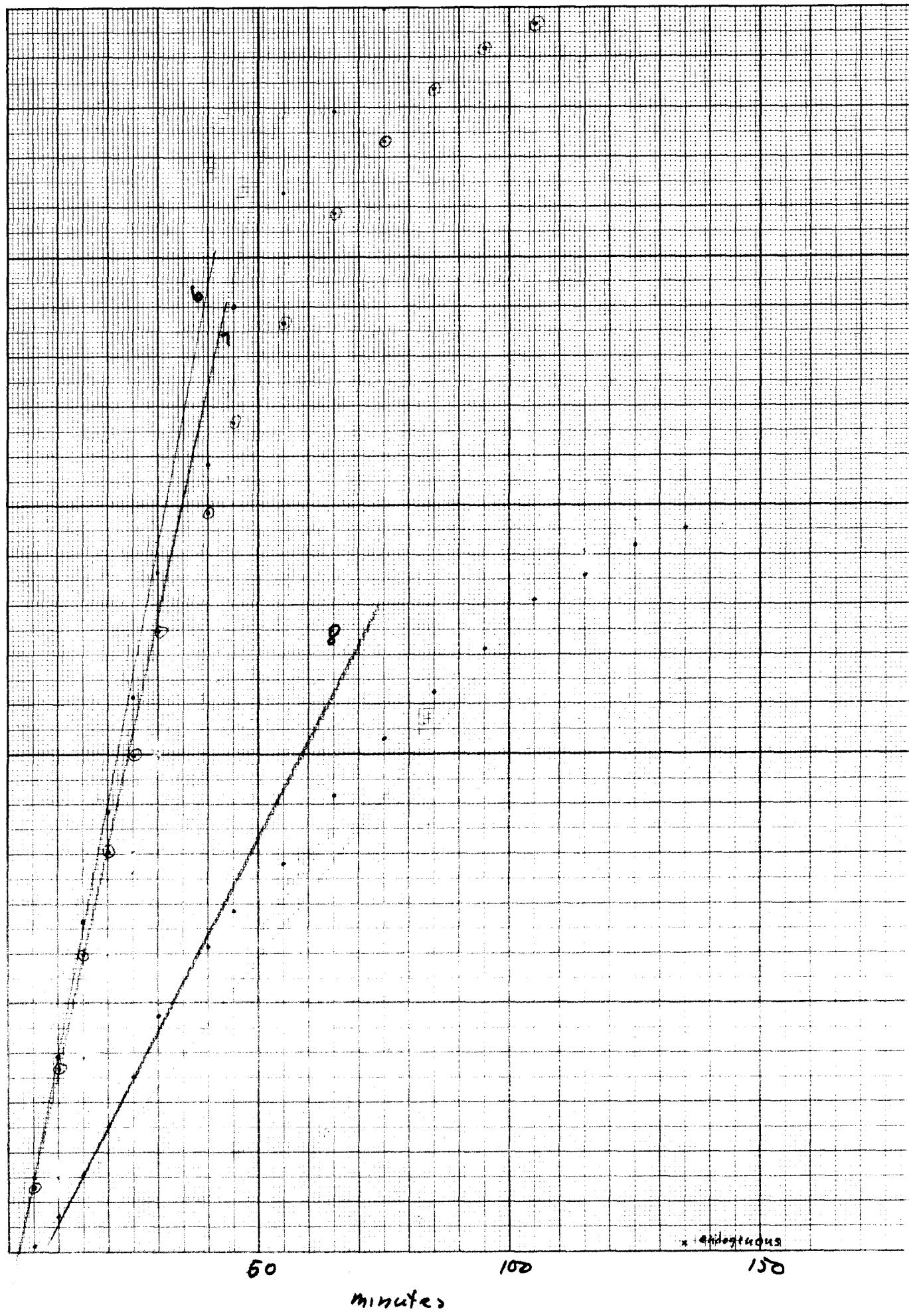
Initial linear rates: mm per hour.  
~~1224~~ 1224 1080 262 1230 1080  
~~1070~~ 1070 390

ml/h. 2007 1825 474 1968 1836 616

N/L = .24 .335

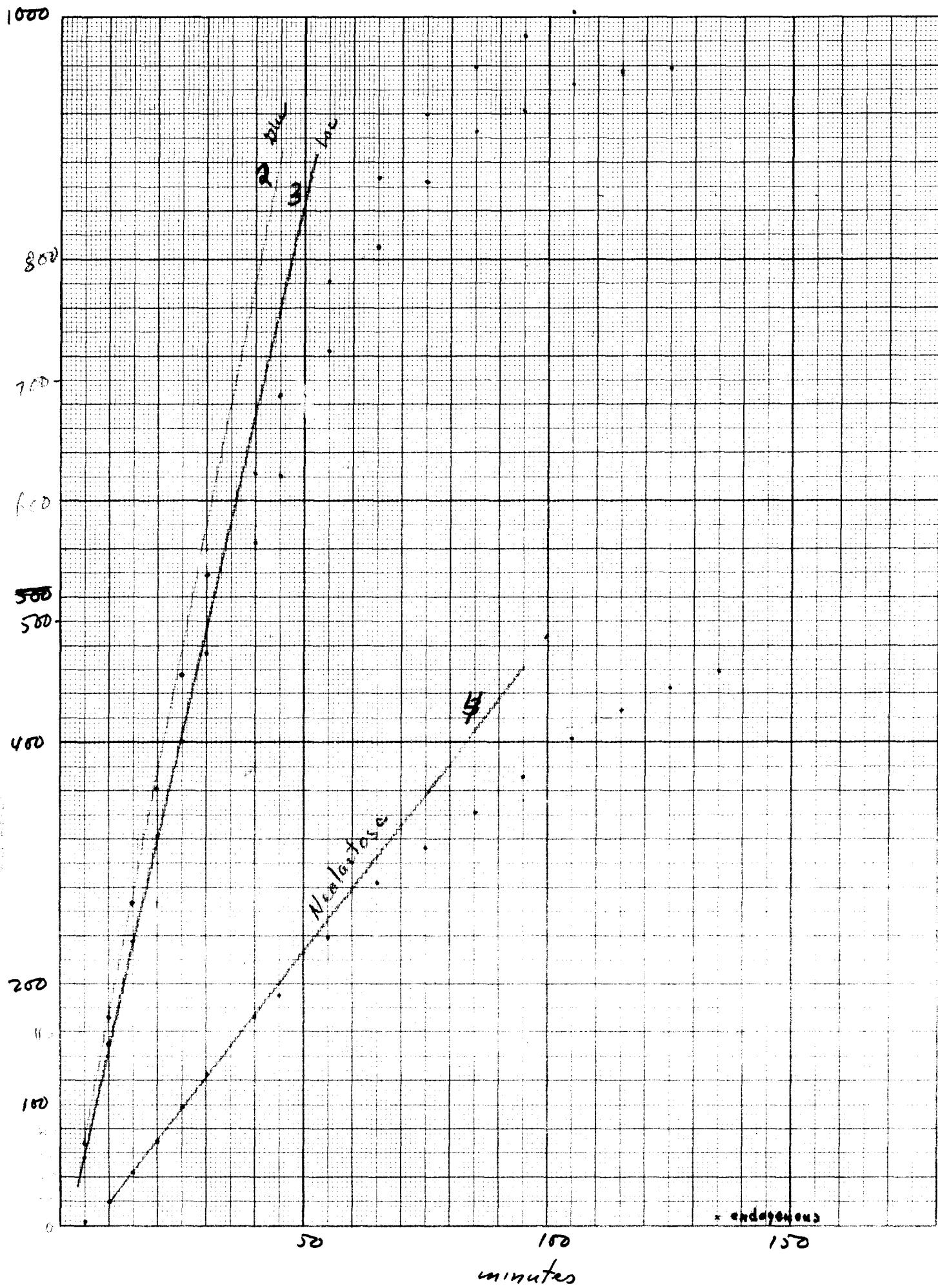
W1301/Lac

730



58-161/Lac

730



# Nodactine response

730A

April 10 (?) 1950.

	Cells	Substrates	Flask
1	3	—	12A
2	3	blu	13A
3	3	lac	5B
4	3	Nolac	2B
5	6	—	10B
6	6	blu	6B
7	6	lac	7B
8	2	Nolac	8B

3-58-161 / Nodactine N2 agar

6 W1301 " " "

barometer  
very low!

	1	2	3	4	5	6	7	8
3 <sup>10</sup>	149	68	45	61	75	59	39	60
3 <sup>20</sup>	147-50	64	45	61	75	9	39	62

TIP

3 <sup>20</sup>	149	64	47-	58	73	4	34	57	62
3 <sup>35</sup>	150	68	68	65	79	12	58	74	70
3 <sup>40</sup>	151	68	83	65	78	11	72	82	70
3 <sup>45</sup>	156	74	108	71	83	17	95	105	76
3 <sup>50</sup>	156	73	121	70	83	16	115	118	81
3 <sup>55</sup>	159	75	143	74	86	17	136	131	81
✓ 4 <sup>0</sup> ?	158	75	120	76	90	22	157	145	83
✓ 4 <sup>5</sup>	164	81	192	80	91	22	189	164	94
✓ 4 <sup>10</sup>	165	82	14	81	92	24	218	175	97
✓ 4 <sup>15</sup>	156	72	181	73	81	14	250	187	88
" 4 <sup>20</sup>	158	77	182	79	87	17	256-67	193-95	
" 4 <sup>25</sup>	158	77	182	79	87	17	119	119	99

98

57

41

250

200

100

50

25

0

10

20

30

40

50

60

70

730A

## Calculations

T	Th		Glu	lac	Molar		Glu	lac	Molar
330	0	0	64	41	2	8	73	4	5
335	5	1	+3	20	6	5	7	21	16
340	10	2	+2	34	5	3	5	36	23
345	15	7	-1	54	6	3	6	54	41
350	20	7	-2	67	5	3	5	74	54
355	25	10	-3	86	6	3	3	92	64
400	30	9	-2	114	9	6	9	114	79
405	35	15	-2	130	7	3	3	140	92
410	40	16	-2	<sup>+32</sup>	7	3	4	158	102
432	62	17	-13	196	-2	-3	7	199	113
445	75	7	+6	265	14	7	6	257	153

8

6

7

62

7

7

6

7

12

9

7

7

17

17

19

9

30

0

x

data in other

run c

run b

See protocol for a  
repetition 730B

Anteros "stable diploid"  
see 727

731

April 10, 1950.

loss 727-1 and -3 as follows, on EMSAC

A	1-	X Y10
B		w677
C	3	Y10
D		w677.

Results of 727-1 and -3 stratified over 99%+

A and C gave good yields, almost all bar+. B, D gave ~~poor~~  
~~moderate~~ yields, bar+ : bar- ca 3 : 1

Picks + and strikes out on EMB Inc.

A: 100+. No distinctive bac<sub>v</sub> but some colonies have lighter, possibly mottled ~~centres~~ centres. Mark these for further purification. Replate all to EMB  
Hal, MHP, Xyl.

3

C. 7A + 40 A.

D 2 testet: holdmusc.

A. Shoot-tests of purified bac + [1-6 excl. J. all xyl + methyl +  
# 20, 21, 22, 40, 53, 54, 55, 56, 93 methyl -  
others methyl +.

2. All Mal ++. All Xyl + #35, 87 Mal - 87 Mal - ?  
other Mal +  
Hold Mal plates in refrigerator.

Troy 727-3 x  
W588

A. 6 restricted on lacEMB as possible frank lac<sub>U</sub>.

Each of these threw off considerable lac- and has appearance suggesting a rather stable lac<sub>U</sub>. } Each is

C. Ditto. C3 distinctly variegated. } pure Mal+

B. 12 EMS Lac+ picked and streaked on EMB Lac Colonies resemble those of A + D. #1, 2 are like C3

D. like B.

Key on EMS Lac

Pick single "4" colonies and streak on EMB....

B:	lac	Mal	Xyl	Mtl
1	++ -	-	+	+
2	++ -	-	+	+
3	++ -	-	+	+
4	++ -	-	-	-
5	v	-	-	-
6	++ -	-	+	+
7	++ v	-	-	-
8	++ -	-	+	+
9	++	-	+	+
10	++	-	+	+
11	++ -	-	+	+
12				

D:

	Loc	Thal	Xgal	MFL		Loc	Thal	Xgal	MFL
1	++ -	-	++	++	13	+ -	-	-	-
2	++ -	-	-	-	14	++ -	++	++	++
3	++	-	++	0	15	++	++	++	++
4	++ -	-	++	++	16	++ -	++	++	++
5	+	-	-	-	17	+	-	-	-
6	++	-	+	+	18	++	-	-	-
7	++	-	+	+	19	✓	-	-	-
8	++	-	-	-	20	++ -	-	-	-
9	+	-	+	-	21	++ -	-	-	-
10	++	-	++	+	22	+	-	-	-
11	++	-	++	+	23	++	-	-	-
12	+-	-	-	-	24	++	-	-	++
25	++ -	-	■ -	-					
26	++ -	-	++	+					
27	++ -	-	++	+					
28	++ -	-	++	+					
29	++ -	-	++	+					
30	+-	-	-	-					
31	+-	-	++	+					
32	++	-	++	+					
33	+-	-	-	-					
34	++ -	-	++	++					
35	+-	-	++	++					

April 18 ca., 1950.

A 58-161 Glu  
B " " "  
C W1301 Glu  
D " " "

2 plates each  $\delta$ ( $\times 2$ ) + glucose 0.1%  
growth in 10 ml H<sub>2</sub>O. .1 ml in 10 ml  
Residue in spot plate for dryn.

A	383	403	(20m)	5.8
B	209	236	"	3.1
C	388	+++		180
D	262	+++		144
-	001	014		

Add benzene to  
each tube and refrigerate

C and D are too active to assay at this dilution. Use  $\approx .01$  ml / 10

				Units/ml	
C'	048	150	(10m)	180	$1u = \Delta = 100 \text{ m}^2/\text{cm}^2$
D'	027	113	"	144	Hydroxyl activation

E, F are suspensions from 4/15. Dilute because fresh suspensions  
1:100, use 2/10 E = 58-161 F = 1301 /no sugar

	Di	Donggi (10m.)	
E	003	017	?
F	004	222	>2000

ca 150 before activation. See  
Maxonite protocol 4/12.

cells 4/15 see maxonite  
picture 4/12  
The "constitutional" lactase differences persist through "activation".

4/20. Qualitative test (spot plate) for galactosidase in W1301 grown in  
 $\delta$  ( $B_2YTRB_1$ ) - various sugars.

Glu	++
Lac	++
Mal	+++
Gal	++
Ar	- ?
MTH	+++
STL	+++
K-Dna.	+ - ?

April 18, 1950.

W1301

A. DN2 — 6 plates Use ca  $\frac{1}{5}$  in 20 ml

B. DN2 Glu 7 plates. Use ca  $\frac{2}{5}$  in 20 ml

Hold 24 hours. Run manometric 4/19. See Protocols.

In this experiment, the cells (B) were tested on a variety of substrates glucose, galactose, maltose, lactose, <sup>but not arabinose</sup>, were rapidly utilized by B. Later that PM, with same suspensions, xylose, sorbitol, mannitol and glucuronate were tested, and were not utilized:

+ A	- B
glucose	arabinose
galactose	xylose
lactose	mannitol
maltose	sorbitol
	glucuronate

This was taken to mean that W1301 is preadapted to sugars A, because previous work had indicated that K-12 was not preadapted under these conditions. But see 4/20.

Cells (A) also utilized lactose and maltose, but rather more slowly in relation to glucose.

Cell density of suspension used: Dilute: .1 / 10 ml

Di	D <sup>10</sup> mpg	Δ	Δ/Di
A 128	370	236	1.84
B 160	560	397	2.48
D <sup>10</sup> mpg	019		

April 18, 1950.

Synthetic Grow 20 hr. aer. 37°. D (Lac or Glu) + BM

		D <sub>i</sub>	D <sub>avg</sub>	L <sup>corr.</sup>	D <sub>i</sub> 208	D <sub>i</sub> 321	D <sub>i</sub> 228	D <sub>i</sub> 332	Δ/D <sub>i</sub>
1	58-161	Glu	016	0330	1	208	-	-	1/10
2	"	Lac	034	190	142	321	44.3	4.43	—
3	W1301	Glu	010	620	585	228	25.6	—	—
4	"	Lac	036	434	385	332	11.6	—	—
				017					

R.A.  
—

5 58-161 Glu 208 200 (20m.) — —

Thus W1301 produces a "constitutive" lactose in very simple medium.

Selection against W1301 is anticipated in non-lactose medium. Transfer successively in D(BMTB.) glucose, lactose, and maltose. Strains out as EMBLac. 1st transfer from W1301/Glu above. 0th: pure lac +

0th: colonies from EMBLac picked to DN2 Glu. Spot test for constitutive lactase:

12 colonies: all oppg+

1: 12 from Mal to DN2 Glu { 58-161 4 colonies  
 4 Lac oppg+  
 4 Glu oppg- .

2: EMBLac - all lac+.

3: " " Test from DN2 Glu to oppg. 4 each: oppg++

*Galactosidase in W1301: Synthetic medium  
with various carbon sources.*

~~734a~~  
734a.

April 20, 1950.

W1301 grown on ~~—~~ D B4TIB, ... overnight in aerator.

Sugar	Di	Dongq	D	$\Delta$	$\Delta/Di^{10}$	R.A.	Galactosidase spot test
1 Glu		269	157	240	15.3		+++
2 Mal		930	146	900	62		++++
3 Lac		372	149	340	23		+++
4 Arab		119	156	90	5.7		±
5 Mtl	0	452	146	420	29		++++
6 Ste	1	875	153	840	55		+++
7 Dna	2	178	160	100	7.9		+
8 Galac		370	168	290	17		+++
0 —		021					

fermentation  
with  $Na_2CO_3$   
at 10m.

Repeat expt. with fresh cells. 0.1/10

	Di	Dongq	1/10	Di	$\Delta^{cor}$	$\Delta/Di^{10}$
1 Glu	018	202		114	197	17
2 Mal		538		223	533	24
3 Lac		147		180	142	7.9
4 Ara		109		208	104	5.0
5 Xyl		179		182	174	9.6
6 Succinic	21	373		120	376	15

as above

Verification: Readaptation of W1301 to maltose, galactose

735

April 20, 1950.

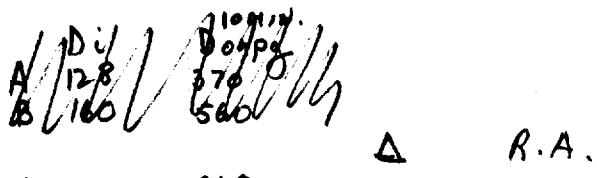
uncon.  
Δ 40m.

Flask	T	Cells	Sidearm	10mg.	A = 58-161	2 plates DN2 blue	10ml.
9B	1	A	-	1	B = 1301	1 plate "	.
2B	2	A	Glu	300			
3B	3	A	Gal	167			
5A	4	A	Mal	202			
10A	5	B	-	1			
6B	6	B	Glu	107			
4A	7	B	Gal	94			
8A	8	B	Mal	80			

Time →  
Flask ↓

	↓ 200 = 210	225	240	317	uncon. Δ 40M.
T	155	152	155	152	154
1	13	11	15	13	-3
2	18	72	203	>310	1
3	43	91	186	217	
4	47	85	162	246	
5	52	52	52	50	
6	54	76	115	158	
7	61	78	111	152	
8	59	77	104	136	

Galactosidase: use .1ml / 10 ml tests



Δ<sub>40mgs</sub> 0.19.

A	319	329	0.23	.072
B	132	273	137	.04

Preadaptive Galactosidase tests  
on E. 2. L. "Suppressor" cultures

736

April 17, 1950.

Brew from EMB colony to Pernasay 10ml. Wash once:

	Di	Dongg	<u>See EZL code</u>
A	010	-	
B	002	-	
C	029	-	
D	007	++	W716 B
E	2D1E 0Y9	±	W716 C

G      017      +++++      Real difference ?? between Lac and Mal  
L      019      +++++  
M      016      +++++

see 734-1

Quantitative reading's interrupted by visitor.

Repeat "0" from DN2/glycer = W716B. in ca 10ml

4/20/	D	Di	Dongg
	-	083	107 <sup>20</sup>

April 20, 1950.

"C" W1301 x W945 nEMS Lac

+, slow, - colonies seen.

Priby .

28 Lac +

2 possible npg - — no! Both are npg +  
Replate on  $\Delta N^2$  gels

12 Lac short

1 possible wpg - But very slow  
Replate and streak out EMBLac

Some purified cultures on NSA slants

Restricts purified bac - prototrophs and holds on to YB Lac.

5/6. Pick separated - and last revisions (corresponding) to  
 $T(s)$ , for test for constitutive lactase

"W1312" is not a prototyph! Repeat cross! (W1301 x W1177  
W1301 x W1178)

ca 50 lac- allowed to revert and lac<sup>+</sup> tested from 0(0) glu  
for constitutive lactase. None were const+.

Conclusion:  $\text{C}_{\alpha}\text{st}$  locus is closely linked to Lac, or  $\text{C}_{\alpha}\text{st} +$  is epistatic to  $\text{Lac} -$ .

TRY: Lac,- on EMB Neolactoce

*Prasagystatum* 6/58-161; W1301

737

A- 58/161

B=1301

April 21, 1952.

Cells	Flech.	0	+ 340	Time				135+
				350	400	410		
1 A	-	B	26	25	23+	28	27	25+
2 A	Glu	A	75+	75+	101	156+	168	257
3 A	Gal	"	70	70	93	127	157	212
4 A	Mal	"	47	47+	64	88	106+	163
5 B	-	"	31	25+	26	29+	27	28
6 B	Glu	"	20	20	58	99	136	242
7 B	Gal	"	23	19	49	84	114+	201
8 B	Mal	"	58	54+	77	101	122	187
Thermobacter.			136+	136	140	140+	139+	140
9 A	Lac	9B	34	↓ TIP	35	40	38	41
10 B	Lac	11A	61		67	97	118	150
Thiom					140	142	137	141
					440↑	450	500(-)	510
								520 39

∴ 58-161 is preadapted to galactose, maltose, glucose but not to lactose. W1301 is preadapted to lactose.

K12?

Y10?

A, B grown on DN2 Agar

C. K-12, 58-161, 1301 for  
preadaptation

138

April 22, 1950.

Flech Cells	Subst	12 <sup>50</sup>	100	↓	105	110	120	130
10 A	K-12	gal	08	09	29	83	174+	297
2 B		gal	50	51+	68	113	179+	202
3 B		Mal	31	29	34	50	88+	147
4 B		Lac	58	59	60+	60	63	67
5 B	161	gal	41+	42	58	114	1209	>>300
6 B		Mal	54	53+	61+	82	140	224
7 A		Lac	51+	52	58+	60	64+	64+
8 B	1301	gal	67	68	76	128+	216	310
9 A		Lac	28	29	30+	83	166+	246+
		Thermostab	134	135	136	138	143	137+

2 plates DN2/Glu/10 ml 2 ml each.

∴ K-12 grown on D(N2), Glucose is preadapted to galactose and maltose, but not to lactose. This speaks for a medium influence since earlier work with cells gave no such preadaptation. Compare the media used!!

Bacterial densities: .1/<sup>9</sup> 4200<sup>9</sup>.

K-12 0.29 Galactosidase spot

- A) 58-161 460 -  
B) W1301 400 +++

Assay A and B for galactosidase. Then add benzyl  
and store overnight.

Separate aliquots. To A; B' add ~~the same~~. Octylale.

Preadaptation of K-12

738

April 25, 1950.

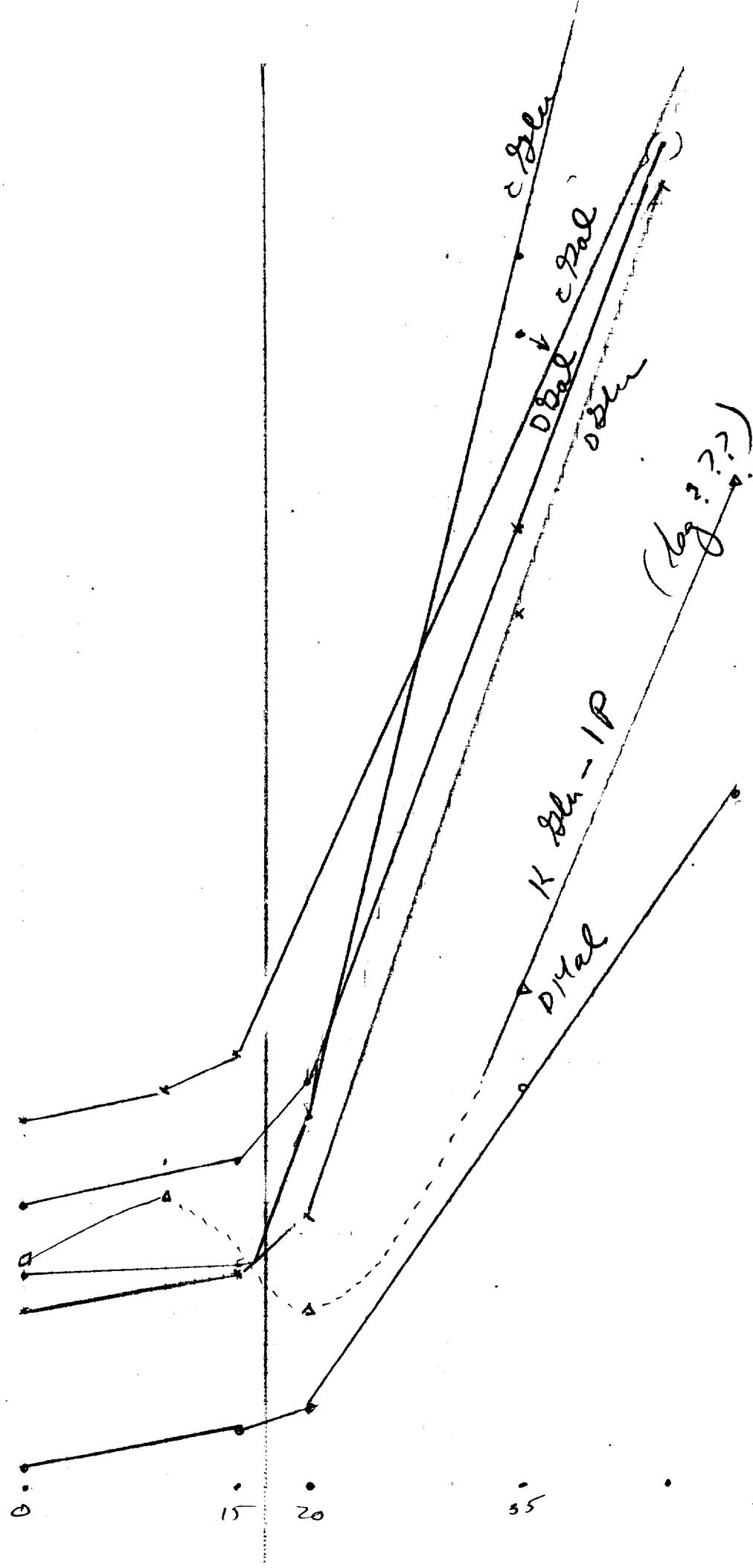
A	Perm.	100ml
B	" Agar	100ml (3 plates)
C	DN2 Glc .1%	"
D	" Agar	"

			15	20	35	45
Flesh Cells	Side 1	Side 2	4 <sup>20</sup>	4 <sup>45</sup>	① 4 <sup>50</sup>	5 <sup>05</sup>
B + A	Glc		06	10	33'	104
A 2 B A	Gal	Mal	22	22	33	95'
6 A 3 B	Glc		27	33	41	113'
7 A + B	Gal	(Mal)	32'	39	43	98'
A 5 B	Mal		37'	44	49	76'
3 A + C	Glc		30	31	52	173
4 A + C	Gal	14 Mal	40	46	57'	162
A 8 D	Glc		25	30	38'	123
B + D	Gal		52	56	61	135
4 A 10 D	Mal		03	8	11'	56'
A, 11 D	<del>Gluc-1-P</del> 5mg (.25 ml)		32	41'	25'	70'
3B/12 TB			135'	137'	137' fluctuating	140 low pressure day!

These data show a clearly constitutive glycolysis of maltose and galactose from E. coli K-12 harvested from Perm assay! They also show glycolysis of glucose-1-phosphate (bearing single phosphate!) (See Leibovitz on metabolism of E. coli)

738a

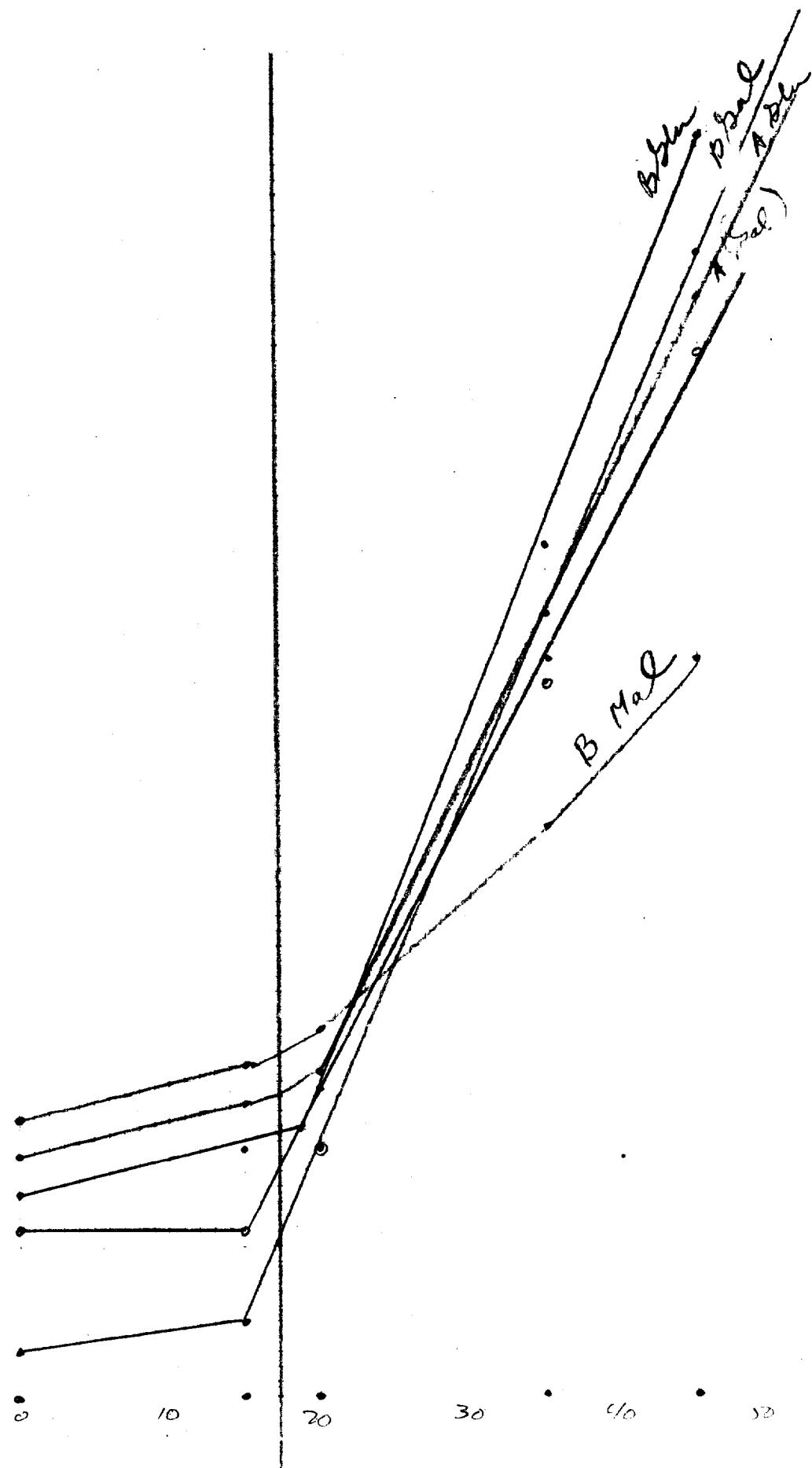
150



>385

Zero

100



## Inactivation of H226

April 25, 1950.

Dilute H226 stocks (from D Lac 4/24)  $2 \times 10^{-6}$ Retain aliquot; UV aliquot 20 sec. at 50 cm. Plate  $\frac{(-)}{0.5} \text{ ml}$   
on EMB Lac EMS Lac. EMB MalA. Unirradiated  $10^{-7}$ B. Irradiated  $2 \times 10^{-7}$ .

A	EMB Lac	V	-	Mal EMB	" + "	Vars	-
1	45	29			9	17	
2	22	18			8	17	3
3	61	37					
4	51	32					
5	16	10					

B EMB Lac (2x)

	V	-					
1	11	27			3	8	1
2	6	36			7	3	3
3	2	12			12	18	4
4	13	27			2	3	1
5	40	23			10	3	0

EMS Lac:

A  $12+ 0-; 33+ 2-$  o Sected.

B

0+	1-
3+	1-
5+	3-
2+	1-
4+	2-
1+	0-
1+	1-
0+	1-

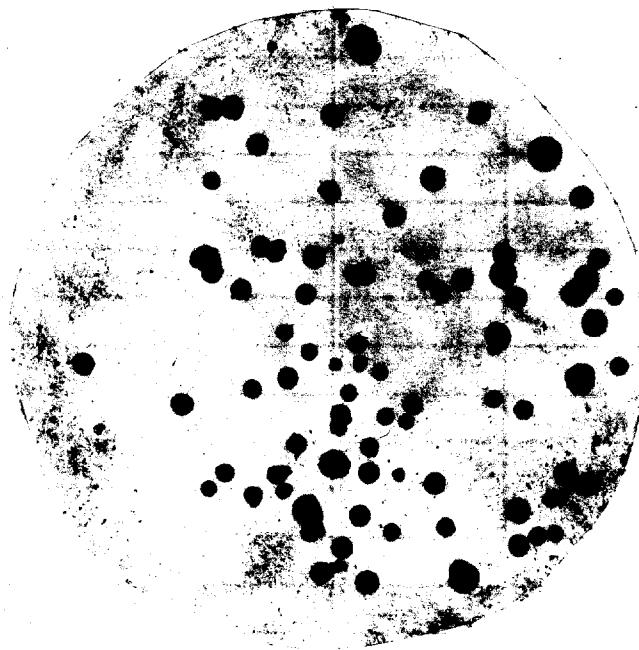
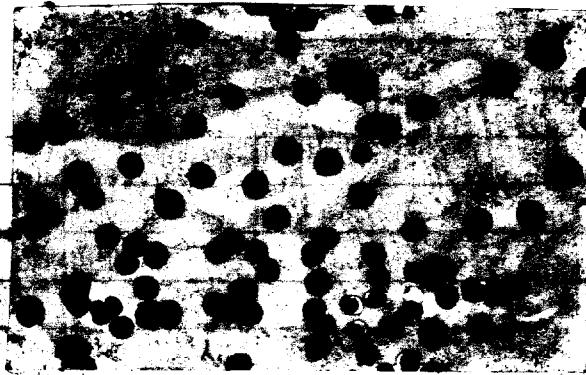
1 sectred.

16+ 10- 1 sec.

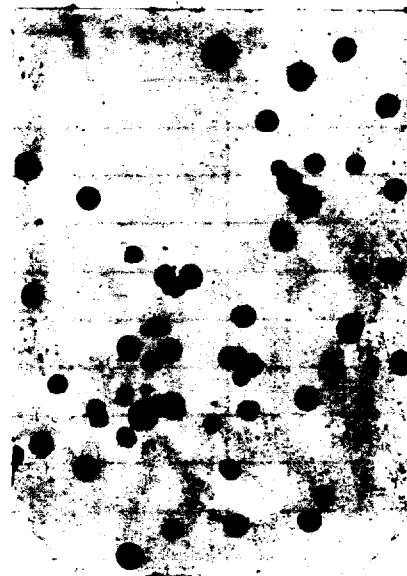
Repeat for larger numbers.



A



B



Inoculations of H226

739a

April 30, 1950.

Dilute to  $10^{-4}$  for inoculation. A. Control. Plate at  $10^{-7}$

B. 20secs uv. Various dilutions.  
("7" =  $2 \times 10^{-7}$ )

Plate on EMBS Lac; Mal EDS Lac; 14al.

A. EMBS Lac

116	8
128	18
115	8
121	19

EMB Mal. 2 plates.

+ and v not distinguishable with certainty  
No - seen.

480      53\* / 533

\* 4, circled, later scored as luc v

B EMBS Lac

B7 24h.

24      58  
47      63  
71      121

All v are delayed.

Mal: see above. All are + or v.

B7 42h.

34      53  
~~#~~52 ~~#~~45

86      98

7396

Jerael. H226

May 3, 1950.

A. EMS Lac	+	-	Mal	+	o
128	0				
130	0				
94	0				
88	0				
<hr/>					
			129	0	
			155	0	
			<hr/>		

B EMS Lac	+	-	sect.
"B7"	33	25	10
	32	20	5
	30	18	6
	46	19	10
<hr/>		141	82
			31

Note "induced" lac - "mutation".

B6: too dense to count well. However, on each of two EM S Mal, no - or sec were seen! Strains out Lac sec on EMS Lac

H226 from A; EMS Lac + were streaked out as EM B Lac, etc.  
Each of 8 colonies was

Mal++ Xglv Malv lacv! This is, then, a partial segregant! Pick to start as 739-1.

Reisolate type H226 from slants.

Kinetics and stability of  
cellular lactose

740

April 25th. 1950

Harvest X-12 from aerated Y2 Lac (1%?) after ca 18 hours  
Wash 2x and concentrate from 100 to ca 10 ml.

Remove ca 2-3 ml and tube with benzene <sup>at room temp</sup> ~~in~~ for autolysis  
overnight. (B). Assay remainder with o-xyg 1/2000 in NaP buffer  
1/50 pH 7.5 (Stored overnight in refrigerator)

On basis of preliminary assay, dilute A 1/50 and B 1/500 to give  
convenient ranges of activity in 20 minutes.

Assay system: 5 ml H<sub>2</sub>O 3 ml 1/15 NaP buffer 7.5 1 ml cells. 1 ml  
substrate (or H<sub>2</sub>O). Add 1 ml Na<sub>2</sub>CO<sub>3</sub> 1/1 to stop reaction. Use drum  
scale of spectrophotometer. Tonic with stop watch, with 30 sec. inter-  
val between additions. Run in 38° "Precision" water bath with motor driven.  
Preliminary turbidity gives variance of tubes. Use volumetric pipettes  
Check, tonic sequence with A. Remove from bath to room temperature when  
<sup>Na<sub>2</sub>CO<sub>3</sub> is added.</sup>

no. of cells	Time	D <sub>i</sub>	D <sub>on pg</sub>		Δ <sub>corr.</sub>	Δ <sub>corr.</sub>
			No. CO <sub>2</sub>	① +1 hr		
1	5	1.00	134	133	0.80	0.74
2	"	0.95	220	220	166	161
3	"	0.98	353	357	299	298
4	"	0.97	458	462	404	403
5	-	0.99	(0.85) (0.81)	0.45	0.38	
6	-	-0.08	(+0.04) 0.00	0.01	0.13	

These data are clearly non-linear !! (presumably  
due to "activation" ~~etc~~ during assay!)

Corrections  
 $\textcircled{1} = \frac{-0.09}{-0.054}$

$\textcircled{2} = \frac{0.21}{-0.054}$

(2) incubate cells in buffer prior to assay (12° to 42°)

April 26, 1950.

	A	Y2 -	B	Y2 0.1% glucose	C	Y2 1% glucose	Agar	1 plate each.	H, C to 7 ml	B to 10 ml	$\text{A}_{20\text{m}}$	
Flask	Cell	Sub	5'	10'	15'	TIP	↓ 25'	30'	35'	45'	55'	substrate
B	1 A	Gal	51	51	55	64	78	89	117	155	91	
A	2 A	Glu	37	34	37	51	68	78	109	150	99	
"	4 B	Glu	63	57	61	76	97	114	161	218	132	
"	5 B	Gal	52	44	47	57	75	91	134	175	118	
"	6 B	Mal	45	33	38	48	54	58	76	101	53	
"	7 C	Glu	54	45	48	71	95	115	166	209	138	
"	8 C	Gal	39	27	32	53	73	92	136	171	78	
"	9 C	Mal	47	36	39	47	46	43	50	37	—	
"	10 B	Glu 1mg	7	-3	-3	7	30	47	106	143	136	
"	11 B	Glu .5mg	23	16	13	20	40	54	114	107	87	=
	TB		169	171	171	176	180	180	189	189	13	

Readings by P. Phaudi.

Note: Utilization of maltose by B but not C. Galactose utilized by each of them! (at a good rate). Experiment needs repetition!!  
Galactose is pseudoptile! (purity of galactose ??)

Utilization of galactose; maltose  
K-12 from Y2

74/a

April 28, 1950.

A. Y2 - <sup>0.1% glucose</sup>  
agar 2 plates } to ml. 2 ml per vessel  
B. Y2 glucose 1% 2 plates } ml. 1 ml 10% substr.  
Both galactose and Maltose  
recrystallized.

Flask	Cells	Substr.	Time →	0:00	10	20	25	30	35	45	100
1A	1	A	-	05	07	07	9	6	8	12	15
2B	2	A	Gal	32	35	35	65	121	191	310	-
3B	3	A	Gal	44	49	47	48	48	48	56	66
4B	4	A	Mal	45	52	50	58	66	81	116	308
5B	5	B	-	37	46	42	44	44	44	50	52
6B	6	B	Gal	16	27	22	55	121	185	296	-
7B	7	B	Gal	46	58	54	53	57	55	61	69
8B	8	B	Mal	54	65	62	65	71	69	73	79
Total				146	156	151	151	154	152	155	157

Thus K12 from .1% glucose ferments maltose ca 1/5 - 1/4 the rate of glucose; from 1% glucose, at only a negligible rate! Galactose is not used significantly by either, perhaps discrepancy presumably due to impure galactose.

May 1, 1950

- W1301 and 58-161, each harvested from 3 plates DN2 2 hr. 1

Wash and resuspend in <sup>30</sup> ml. 1/20 NaHCO<sub>3</sub>. Fleets 2 ml  
cells, .1 ml 10% substrate in sidearm.

		0	5	+ 10	15	25	30	35	45
1A	-	161	55	57'	59'	57'	61	64	62'
2A	Glu	161	48'	50	61	93	153	183	209
3A	Gal	161	13	10	10	17	20	20	23
4A	Lac	161	51	52'	51'	55'	59	58'	61
6A	Lact	1301	60'	65	63	70	71	72	70
5A	Glu	1301	62	64	77'	119	181'	206	236'
7A	Gal	1301	76	74	70'	84	82'	82	84'
8A	Lac	1301	24	27	28	69	127'	150	177
	ThBar		133	135	132	138	139	136	140

W1301 is preadapted to lactose but not to galactose. Galactose should be accumulating! It uses lactose as fast as glucose.

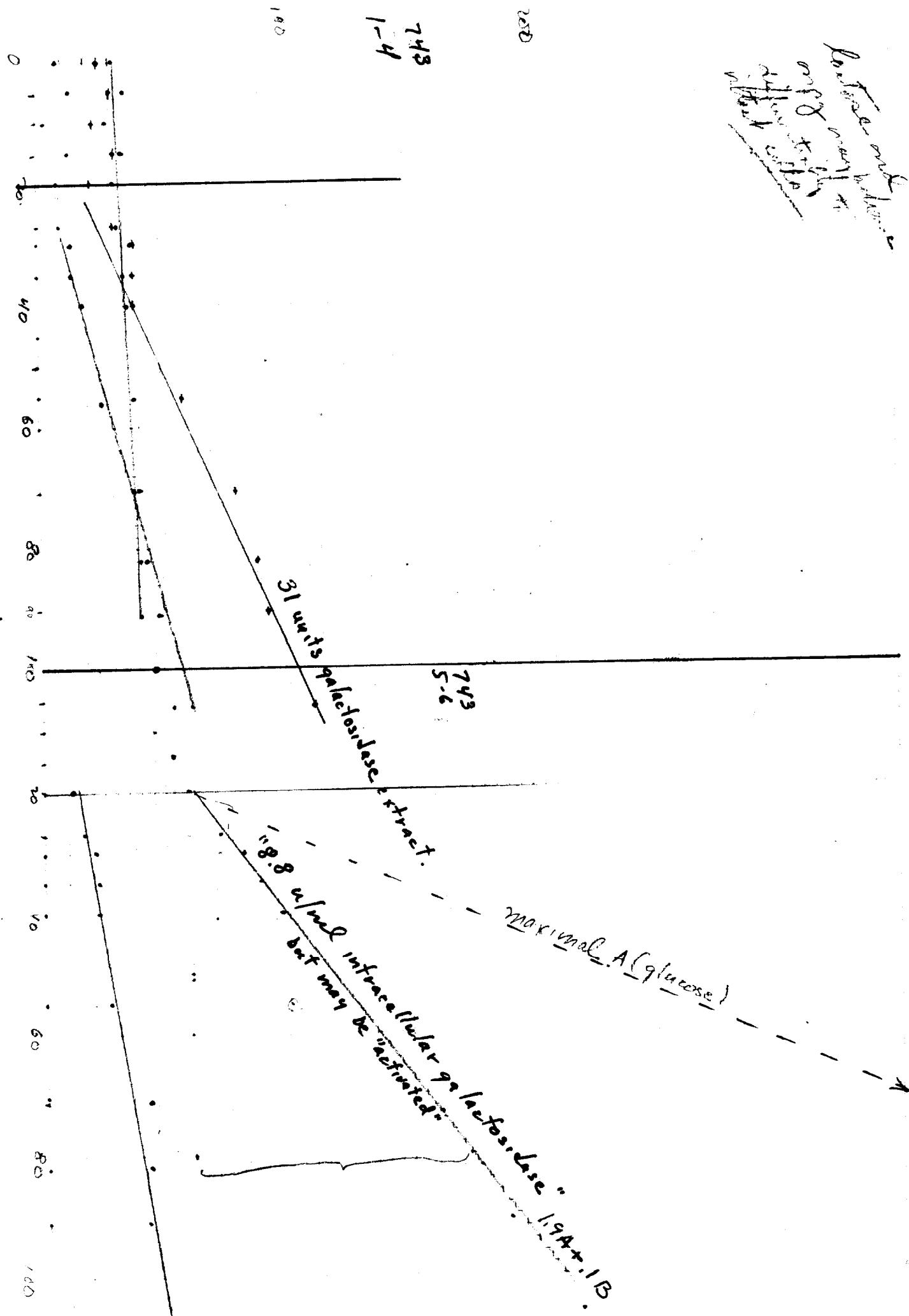
1/10	Di	Dong	A
A	252	260 <sup>10M</sup>	-
B	249	6 MIN <sup>473</sup>	ca 220

∴ B shows an activity of  
 $\frac{4 \times 2.2}{2} = 88 \text{ u/ml}$ .

(Test by B, exposed to conditions of Warburg vessel.)

Judging from efficiency of B in mixture with A, B contains approximately 7x the amount of lactase needed to keep pace with glycogen, assuming equal potency within and outside the cell.

for  $\alpha$ -galactosidase  
on S. typhimurium  
different dilutions



## H243 Segregation pattern

743  
b

$$\begin{array}{ccccccc}
 \text{Gal} & - & \text{Mal} & - & \text{Mgl} & \times \text{gl} & - \\
 \hline
 & + & + & & & + & + \\
 & & & & & & \\
 \text{= predominant}
 \end{array}$$

All -	108
All +	6
Bad - + +	15
Bad + - -	5
X- / / x x x	
✓ 7.0	2

Segregation of H243

743

May 1, ... 1950.

Picks ~~#~~ colonies of H243 from EMS Lac and inoculate into  
~~#~~ Dilution Penassay. After 48 hours, plate out on various media:

(1)

7	EMB Lac	142 total. 4 clear lac <sup>v</sup> . A number ( $\pm 5$ ) of others are faded lac <sup>v</sup> reminiscent of lac-Lac <sup>v</sup> .
	EMS Lac	3+ 0- (some minute hold)
	EMS Gal	7+ <u>1-</u>
	EMS Mal	2+
6	S Lac	12+ well defined. several small - hold.
	S Gal	11+ "
	S Mal	18+ 6-
5	SL	++ numerous poorly defined -
	SG	ca 20 -
	SM	30 -

(2)

7	BL	196 <u>total</u>	1 lac <sup>v</sup> .
	SL	0	
	SM	0	
	SG	2+ ? - hold.	
6	SL	7+	Many small -?
	SG	12+	" "
	SM	1+	
	BL	3+	Many -.
5	SM	181+	31- 2 sec.
	SG	26-	2 sec?

(3)

7	BL	84 tot.	2 X.
	SL	2 tiny +	
	SM	2+ <u>1-</u>	
	SG	1+ 1-	
6	SL	13 +	Many small
	SG	25+	4- " "
	SM	23+	1-
5	SL	Many large +	
	SM	194+	75- ✓
	SG	184+	62-

Segregation of H243

743  
a

May 3, 1950.

(4)	7	BL	<del>SV</del>	SV	(1 faint)
		SL	4+		
		SG			
		SM			
	6	SL	11+		many small
		SG	18+	1-	many small.
		SM	11+	7-	
	5	SL		12-	
		SG			
		SM	147+	33-	

Partial  
segants. Pick EMS: Gal - and ~~Mal~~ Mal - to EMB; ~~lac~~ Lac for  
partial segants.

(4) Gal - : 6 tests 2 Lac +

Mal - : 22 tests 2 Lac +

Repick lac + and streak out  
on EMS lac, EMS lac, Mal, Gal  
for verification and reseleccs.

(5) Gal - : 33 tests 9 Lac +

Mal - : 37 tests 8 Lac +

Segants: Pick lac + at random and test as four segregates:

(1) Mal Gal Xyl MFR

# Partial segregants

May 6, 1950.

Testicals possible Gal- or Mal- Lac<sub>-</sub>, from EMS Lac +.

③	EMS →				EMSLac	Lac <sub>-</sub> Mal <sub>-</sub> Gal <sub>-</sub> Stac			
	Lac	Mal	Gal	Stac		Lac	Mal	Gal	Stac
1	+?	+-	V(+,-)			9	+ (?-)	+	-,+
	V	V	V			10	V	V	++
	V	V	V			11	V	V	V
	V?	-				12	V	V	V
5	+ (v?)	+-				13	V	V	V
6	V	V	V			14	V	V	+v
7	V	V	V			15	V	V	V
8	V	V	V			16	V	V	+v
						17	V	V	v

(#4, 5, 9) should be tested again. How can the - appearance be accounted for?  
Each appears to be

④	1	V	V	V	Lac + pure	{ probably many fact crossovers.
	2	V	V	V	Gal -	
	3	V	V	V	Mal -	
	4	V	V	V	B <sub>1</sub> -	

H242 Recovery (Mal+ - purified and retested):

1-8: pure Mal+ app. Lac - (i.e., segregated) but hold.

Repicked Gal and Mal- from EMS (#1, 3, 4) (ca 3 each)

Gal EMS	Gal - all -	Mal - 7+ 7-
Mal EMS	12- 1+	13- 1(-+)
lac EMS	all -	

May 1, 1950

6 plates 1/16 to 10 ml. 9 ml. dieldrinite P.D.S.

1 ml = dilute to 10.  $(\frac{1}{10})^2 = .01$  plate / 10 ml = 1/1000 dilution  
2 parts incubated under bungs

Day 16 May

Extracting 19.5% tetra algin of suspensions of dry cells (24%)

flor 4. Soil super viscous & elastic.

*Meloe proterus* occurs in some countries.

use of an electronic device.

*Infected cells.*

Dry cell script

Benzene all signs

Note very low intact & total pollen by here day two

Add ~~longer~~ to align ~~as~~, 5

*Estimate* ~~days~~

12949 A

卷之三

3 249

Di  
192  
022  
~~7~~ 759

Dairy  
500  
358  
H70

20m.  
5m.  
5m.

$$\text{ca } 10 \times 3 = 30 \text{ u/ml}$$

① mg equivalence of bacterial density unit. Let 1 BDU = quantity of bacteria giving opt. dens of  $10^{10}$  in 10 ml.

Then 276 mg dry cells were obtained from 9 ml of a suspension which had a density of  $10 \times \frac{1}{1} \times 1.39$  per ml =  $9 \times 139$  BDU.

$$\therefore 1 \text{ BDU} = \frac{276 \text{ mg}}{9 \times 139} = \underline{\underline{217 \text{ } \mu\text{g.}}}$$

Discard this expt.

Manometric efficiency

74%

May 6, 1950.

Cells harvested from D V2 1% Gluc (stored 24h.) 200 ml agar.Suspend in ca 40 ml NaHCO<sub>3</sub> 1/20.

Dissolve 2 ml per flask. + add cells.

1	hole	Vessel.	Sidamor (10 mg.)	TIME →	0	10	35	↓ 40	45	50	55
A	1	g	-	33	34	39	37	43	43	41	
A	2	g	glucose	17	-02	(18)	06	(15)	103	167	219
A	3	g	lactose		42'	42	50	52	51	48	
A	4	g + .1ml A	lactose		48'	46'	54'	60	64	66	67
A	5	g + .1ml D	lactose		24	23'	30'	34	37'	37'	37
A	6	.1ml A	lactose		23	22'	25	24'	25	25	25
A	7	.1ml D	lactose		34	32'	35	38'	38	35	32
9B	9	TB	Bac		44	43	46'	49'	48	45'	43

1	65
2	38
2 [217-7]	78
3	47
4	74
5	41
6	26
7	32
TB	44

Inadequate lactose!

May 5, 1950.

200 ml DN2 loc 1% K-12 harvested. Wash; suspend sediment in 10 ml. Dry 9 ml aliquot; Dilute 1 ml to 10. Treat ca 2 ml of A with Benzene (B). YDU -

Preliminary assays (see 744.)

A: 30 u/ml. Dilute 1:15

B:  $50 \times 4 \times 3$  units/ml, ca. = 600 u/ml. Dilute 1:200

D: 430 mg dry cells (two staged drying).

$$\therefore \text{Calculate } 1 \text{ BDU} = \frac{430 \text{ mg}}{9 \times 192} = .248 \text{ mg} \quad (\text{cf. 744/217})$$

Assume about .23 mg.

- D) Prepare a 2% suspension of dry cells. Remove aliquot first and dilute 1:10 (2 mg/ml) (D) <sub>(2x incanti.)</sub>
- X, Extract remainder 3 hours and repeat sediment. (Reextract = X<sub>2</sub>)  
Yellow, viscous, opalescent supernatant

Cells

(744a)

Kinetics of ~~t~~<sup>un</sup>-treated cells. Dilute ~~to~~ 1/15

Final conc.	Con A	Con B	$\Sigma$ Con	$\Delta \frac{A}{V}$ 3.92	$1/V$	$1/S$
1 M/500	422	79	042	121	301	33.2
2 M/1000	339	79	021	100	239	41.8
3 M/2000	260	79	010	89	171	58.5
4 M/5000	177	79	004	83	94	106
5 M/10,000	140	79	002	81	59	169
6 0	079					
7 M/1000 No cells	021					

(would be 2.2  
for linearity!)

Terminate with  $\text{Na}_2\text{CO}_3$ . Read within a few minutes.

After 20 minutes 37° Cells fresh (afew hours in  $\text{H}_2\text{O}$ )

NaP M/50. 10 ml volume + 1 ml  $\text{Na}_2\text{CO}_3$ .

Volumetric Quant Technique

$$K_s (\text{cells}) = 6.3 \times 10^{-4}$$

$$V_{\max} = \frac{1}{25.5} = 392 \text{ (3.92 units/ml suspension.)}$$

The cell suspension ~~estimated~~ was  $\frac{1}{15} \times \frac{1}{10} \times \frac{1}{9} \times 430 \text{ mg/ml}$

$$\text{(Calculated density unitage : } \frac{3}{3} \times 192 = 128 \text{ )} = \frac{430}{1350} = 318 \text{ r/ml}$$

$\approx 318 \text{ r}$   
in perfect agreement, as demanded! )

Lower O.O. due to alkali).

Therefore  $V_{\max}$  was  $3.92 / 318 = 12.3 \text{ u/mg.}$

Kinetics: Benzene treated cells.

7449

May 5, 1962.

B)

Dilute ~~to~~ 1:200 to place in convenient range for assay.

M/-	Doupp	Conc	A	1/A	D'	Conc	A'	1/A'
500	482	049	433	23.1	490	039	451	22.2
1000	460	028	432	23.1	461	022	439	22.8
2000	403	017	386	25.9	400	013	387	25.8
5000	293	011	282	35.5	300	008	292	34.2
10000	200	009	191	52.3	197	006	191	52.3
		007			004			
H <sub>2</sub> O					002			
M/500 extra					033			

See 744 for correction factors.

Repeat readings in using a single tube D'

$$K_s = 1.25 \times 10^{-4}$$

Note difference in Vmax : Cells are  $\frac{200}{15}$  conc

$$V_{max} = \overline{21.0} = 476$$

$$\text{Absolute activity} = \frac{476}{392} \times \frac{200}{15} \times 12.3 \quad 199$$

744a cells

180<sup>o</sup> C

He

180

185

190

195

200

202

204

206

208

210

212

214

216

218

220

222

224

226

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May 1, 1950.

Dry cells of K-12 harvested from ca 100 ml Y2 Lac,  $P_2O_5$ , non- $\text{K}_2HPO_4$ .  
Yield: ca 35 mg. Titterate and sheleve in 3.5 ml  $H_2O$ . Sediment and  
retain supernatant.

- 77 u/mole
- Preliminary assay in 0.5 ml of extract (rather weak! - no previous salt treatment?)
  - optical density of 742 A cells (for assay!)

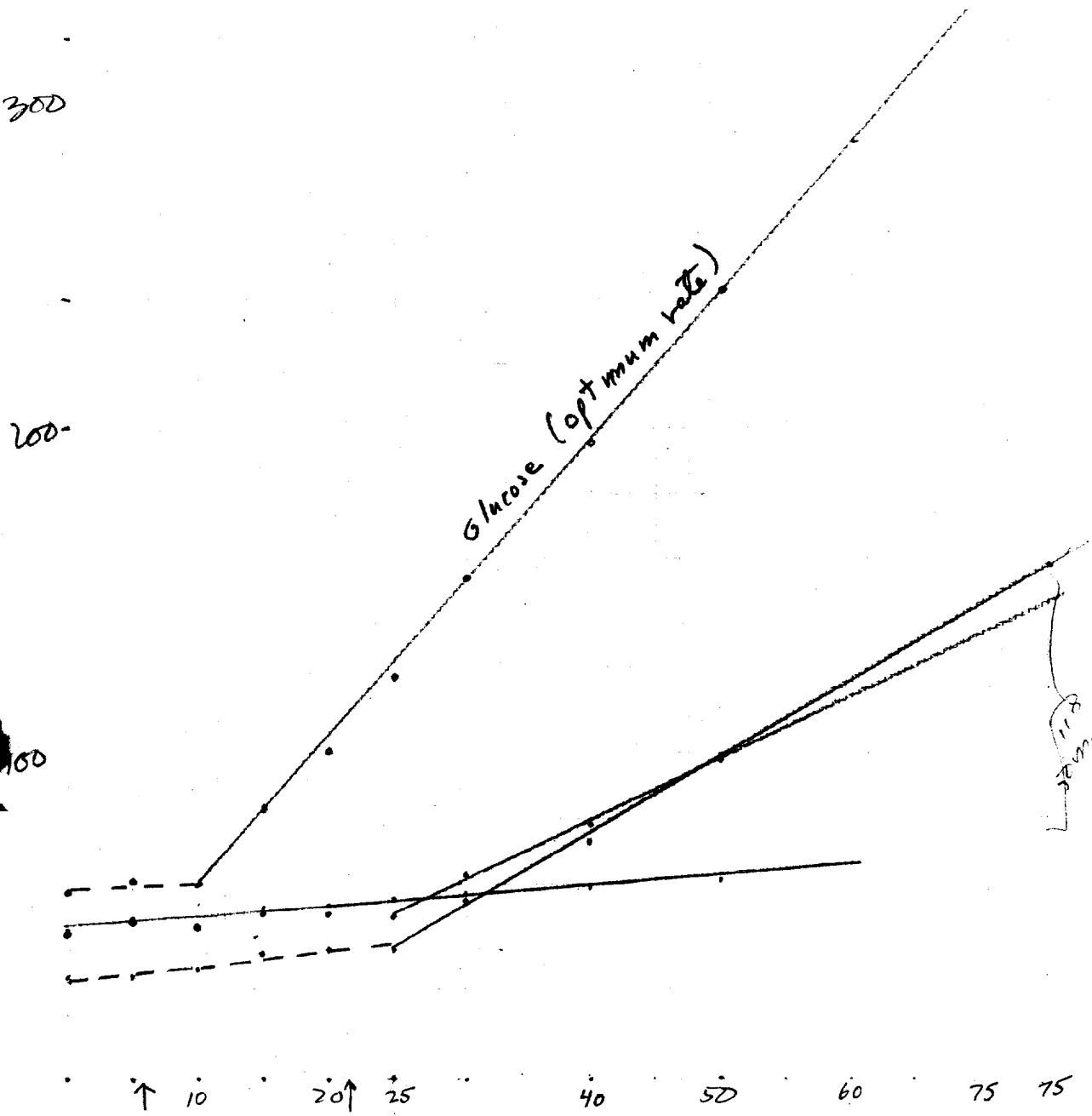
See 742

Use 2.0 ml 742 A cells. Turn →

Side 1 Side 2

	0	5	10	15	20	27	30
1 lactose 10mg	31'	36	28	35	32'	33'	38
2 " " 2ml	8'	14	3'	13	8'	09'	14
3 " " 4ml	25'	30'	23	32	22	31'	39
4 glucose 20mg 4ml	28'	35	29'	38	32	34'	40
5 1.9 me $\alpha$ -lactose	46	54	46'	53'	59'	72	82
6 1.9 me $\beta$ lactose	8'	18	7	16	12'	16'	21
T.B.	157	162	156	162'	160	160	163'
	35	40	55	71	82	91	106'
1 35'	37	39	41	41	41	47'	
2 14'	18	26	39	43'	48	61	
3 39'	39'	58	80	88'	93	112	
4 37'	37'	36'	42'	42	37	40	
5 88'	97	101'	161	177	189	217	
growth? 6 22'	22	26	42	42	61	47	
T.B. 164	162	161	169'	168	163	167	

745A



Parametric assay of lactase

745 A.

May 4, 1950.

Use same cells (742 A!) for assay of glucose liberated.

Cells 2ml	Sideratin <sup>long, 2 ml</sup>	2	0	5	↓	10	15	20	25
			46	49'	lac	47	53	54	55'
C 1 A	Lac	—	58	61	glu	60	83	101	124
A 2 A	glu	—	744	745	utn	45	51	51	50
A 3 A	Lac	744 <sup>1 ml</sup> utn	745 "	48	utn	34	39	40	lac ↓ 40
A 4 A	Lac	" 2ml	31	32	utn	34	39	40	lac ↓ 40

31	40	50
57	59	61
157	196	243
63	78	98
55	73	99

Glucose A = 2.28 / 50 min

lac +

Lactase A = 11.8 / 50 min.

(opt.)

Should steady state be reached at a suboptimum level?

# Thioguanine Efficiency

746

May 8, 1950.

3 plates

2 plates

Harvest K-12 from DMR, 1% Glu(G) and Lac(L)

<sup>5 ml</sup> Resuspend in ~~and~~ <sup>the</sup> NaH<sup>103</sup> buffer.

↓ cell density computed (from opt. density)

Resuspend in 20, 15 ml respectively.

Optical density: (.1/10)  
<sub>cuvette</sub>

	O.D.	mg/ml	Doupg
G	360	8.3	<sup>1.60 mg</sup> 1.60 mg
L	291	6.7	<sup>1.40 mg</sup> 1.40 mg

Use 1 BDU = .23 mg as conversion factor

Repeat      <sup>.03 ml/10</sup>  
L 091

122 !

very low activity!

Need of NaH<sup>103</sup>? . ?  
or no adapt taken

## Effects of Bicarbonate buffer.

### Manometric assay.

746 a

May 9, 1950.

Cells harvested from 2 plates each D<sub>1/2</sub> Glc; Lac (.1%) and resuspended in 10 ml H<sub>2</sub>O. From "L"; aliquot A diluted 1:1 with H<sub>2</sub>O; B with 1/10 NaHCO<sub>3</sub>. Let stand (under CO<sub>2</sub>) at room temperature for ca 3PM - 8PM. . . assay in 1/50 NaP 7.5 . . .

Assay (.1/10)		20	T → O	5	10	15	20	30
Pataquitos	A	207	Dougs	22	22	22	24	28
and me	B	191	271	25	24	30	42	59
	-	-009	012	45	47	52	59	74
				25	28	25	30	30
				65	69	68	64	71
				54	59	59	56	60
				121	127	126	124	131
				TB				127
Trial B (Lactose) cells.								
B	B 1	Siderum		40				
	B 2	Lac		41				
	B 3	blue		114				
	B 4			107				
A	A 4	Acells-blue		25				
	A 5	" Lac		66				
	A 6	A <sup>2nd</sup> blue lac		54				
	A 7	A blue lac		TB	122			

No bicarbonate effect, but these cells have very low lactase activity!

# Irradiation of H226

747

May 9, 1950.

A - Control  $10^{-7}$  H226 resolated from single colonies.

B - UV 20 sec. Delutions indicated. Irradiate the  $10^{-4}$  dilution.  $\phi^{30} \rho 14$ .

$$\underline{\text{"7" = } 2 \times 10^{-7}} \quad \underline{6 \times 10^{-6}} \quad \underline{6 \times 10^{-5}}$$

## EMB Mal

A.	+	-	v	Some "7" may be v.
	19	1	131	
	10	3	122	

B	64	20	142	Not accurately countable.
	87	28	146	Other plates not counted

## EMB Lac

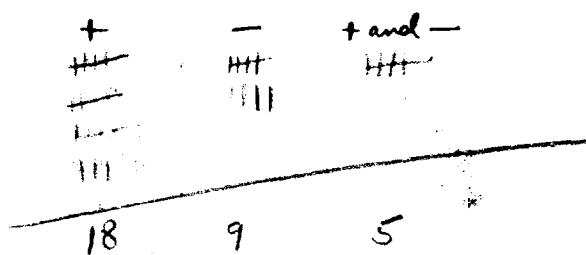
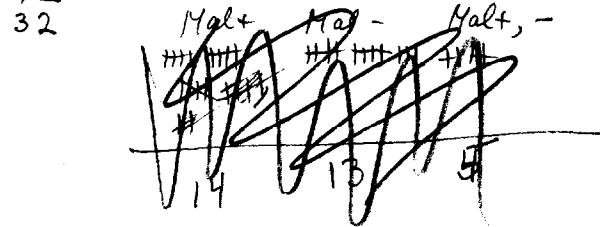
A	v	-	
	188	17	
	212	10	
	<u>400</u>	<u>27</u>	
	<u>200</u>	<u>14</u>	

B	127	108	Many colonies ○
	134	80	
	80	128	
	<u>341</u>	<u>316</u>	
	170	162	

Picks Mal - colonies and test for Lac v. See 147A.

Picks whole lac - colonies and streak out for Mal + / - content.

~~tests~~



B2 did not grow on synthetic (EMS)

A and B1-3 appears to be Mal-Lac.

See 747D

H226 : UV.

747A.

- a) Pick apparently pure ~~Mal-~~ Mal- colonies to EMB Lac, for "partial segregants". (1, 2, 4)

A] 4 Mal "-" : 3 Mal-Lac- #3: Mal-, +? ; Lac - +? Restructure (747-A)  
has -v in both lac and mal REISOLATE

B] 38 Mal "-". 13 of these have apparent Mal+ in break, usually not so clearly evident lac+.

3 possible Mal - Lac v. Restriction: 747AB-[1-3]. May be  
has Mal - Lac +  
agglutinins for  
some colonies  
Remaining 22 are apparently pure Lac - Mal - .

Picks from Hal sectored colonies (EMB), the - as clean as possible, and the + correlate. Brush on EMB Mal; fac:

Pick Mal-treatments from c) and check on XylemB

$$19Xg + 17Xg = 1 \quad (\text{High crossover frequency?})$$

40 \*

# EMS Platings

747B

May 12, 1950

60 hours.

EPS	+	-	sectoral
74	0		0
101	0		0
140	1		0

EMS 14aL	49	0	0
	155	0	0
	141	2	0
	134	1	0

E145 Lac	101	15	12
	82	9	16
	101	13	9
	98	23	20
	120	15	11

Mal	102	4	11
	89	6	10
	65	5	14
	65	5	3
	102	4	12

5/22. Pick - and sectorial -, +  
from HalEMS to lacEMB, lacS,  
and HalB.

Studs \* from EMS Lac to  
EMS Mal. 6"- " and 7"  
pairs (sector)

Analysis of these plates was interrupted (by weekend vacation, trip and lack of assistance)

Experiment to be repeated on more appropriate scale. Test for:

- a) auxotrophic lac; Mal
  - b) Partial segregants in intact and sectioned Mal- (E73) colonies.
  - c) Carefully examine "inverted haploids" for trace residual diploids (cf. 147A-[B]).

UV-induced partial segregants

747D

May 16, 1950.

1-3 = 747B-B1-3 4 = 747B-A1

- a) from EMS (excludes 747D2)
- b) from EMB Lac.

	EMB Lac	EMB Mal	EMS Lac	Mal Xyl 14L
a.	1 V 2 - 3 V, - 4 V	- +,-, v? -	+ +	not part. seg.
b	1 V 2 V, - 3 V 4 V, -	- V V -	EMS Lac + no growth + +	(Methionine less) not part. seg.
				V V - V + - -

5/18 Repick 3 single Lac<sub>v</sub> colonies from 2a, b. Restreak on EMB Lac; Mal and determine mutation: Methionine less.

Repick single Lac<sub>v</sub> colonies to Mal-EMB to obtain reversions, i.e. hemizygosity test.

SUMMARY:

#1.	Lac <sub>v</sub> Mal-Xylv M <sub>R</sub> v	Prototroph
#4	Lac <sub>v</sub> Mal-Xylv M <sub>R</sub> -	Prototroph
H244 #2	Lac <sub>v</sub> Mal-Xylv M <sub>R</sub> +	Methionine less.

PROJECT:

1. Hemizygosity of Mal- in 1, 2, and 4
2. Identification of triploids in outcrosses of #2.

5/24  
747E. Autocross <sup>a</sup> segregants of H244 ( $Xyl^-$ ) to Y10.  
M + Lac - Mal - Xyl - Mtl + x Y10  
1.

## H244 Reversion heterozygosity test

#2. Segregation: streak out on EMB Lac <sup>lac+</sup>.

a) Test  $\frac{5}{-}$  lac - on XylEMB and DMTLB, } a' Test  $\frac{5}{\text{Xyl}}$  - Lac - Each was  $\frac{+}{-}$   
Each was M- Xyl+

5/21/50. b) Test addnl. lac - on XylEMB: 34 Xyl+ 15 -.

Check Xyl + with  $\frac{+}{-}$ .

H244M+

c) Brush on MalEMB for reversions (heterozygosity tests): 3323 Brushes.

5/21 3B papillae picked from these and streaked on EMB Mal, Lac

\*1,4. ~~Brush on EMB Mal for reversions.~~

5/22 Were predominantly + or - on Mal and Lac, repeat single <sup>Lac+</sup> to Mal ~~+~~ for verification as possible Lac+ reversions.

5/23 27 groups sampled. Mostly Mal-. 4 groups had  
~~+~~ Mal+:  $\frac{1}{4}$ ;  $\frac{1}{4}$ ;  $\frac{3}{6}$ ;  $\frac{1}{3}$

Re streak each of these on Lac, Mal EMB, and re streak single Lac+ colonies:

	Lac	Mal	Mal-	Mal+	Pick - to EMB Xyl. M+ may be
1	- v	+ v -	4x- 1x±	9x+	
2	v	+ v -	5x- 1x+	7x+	{ CIS }
3	v	+ v -	2x- <del>+</del>	1x+	
4	v	- v	3x- 5x+	2x+	TRANS??

May 8, 1950.

A	x 1272	} EMS lac	Pick 100+ from B and streak out on EMB Lac for Lac <sup>r</sup> . Yields very low on A. C considerable.
B	x 1178		
C	x 340		

c) 73 Lac+ picked to EMS Mal. All +.

c) v. good yield. lac+ and lac slow (incubator at 36° - threshold for Lac<sub>3</sub><sup>r</sup>).  
20+ and 20sl plated to water susp.

and spotted on EMS lac, B lac, ~~A~~ glu

20 Lac+ : ~~all~~ glu

glu

20 lac sl : ~~all~~ - . Are any of these const Lac+? (at 30°?)

Tube to W252 for further test. Picks from EMB Glu for spot plate tests with on pg.

glu+ 1, 3, 4, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, ~~20~~ 20 70%

ng+ 2, 5, 7, 9 12, ~~19~~ 19 30%

" 12

The ng+ glu- cultures must represent the genotype  
const + Lac<sub>3</sub><sup>r</sup>. Purify to verify temperature behavior. Streaks out  
the two types. Pick from EMS lac.

~~Inoculate NSB with # 11, 12, 1301 and 58-161. Incubate at  
30 and 40°.  
of 16 glu+ cultures, 10+, 6- on ng.~~

See 749!

Test various suppressor stocks for constitutive lac operon (escape from blunting)  
W252: very strong+++ on ng.

7483

May 11, 1950.

B

100 Lac+ picked and streaked on E9B Lac.

1 likely; 2 improbable lac<sub>v</sub>. Picks + over colonies and streaks  
88 further lac+ picked and streaked: 1 definite lac<sub>v</sub>.

of previous set, # 1 is lac<sub>v</sub>, others probably not. Keep as  
748B1 and B2. Brush as DN272m for 5th test.

On pg spot tests: Both B1 and B2 any constitutive.

C:

Suggetions of Col among ~~lac+~~ lac+ (from EMS spots to DN2  
on pg spot plate tests)

1-12 5+ 7- (5, 6, 9, 10, 12)

These data not very informative.

#11 (upg+) is lac+ at 44°

Both are blue -

#12 " lac- "

Constitutive lactase suppressors

749

May 11, 1950.

Test various suppressor stocks for constitutive lactase:

a) scrape growth from (old) slants for npg spot plate:

W	npg	Constitutive	
251 a	±	W108 Lac <sub>1</sub> + Lac <sub>3</sub> -	(faints Lac + Gal) L+M+D-
2 252	+++	W108 Lac	L+M-D-
3 327	-	W108 "	M+ L- D-
4 329	-	W108 "	M+ L+ D-
5 349	±	58-161 Lac+	
6 716 D	+	Y70 Lac,- Suc+	
7 716 E	+	" "	
8 1301	++	58-161 Lac+Const+	

b) inoculate into Pernassey and incubate 11AM - Assay.

After 36 hours, spot plate test with one drop of yeast culture

251	-
252	+++
328	-
329	±? Repeat:
349	-
396	-
397	-

# Lactase economy

750

May 13, 1950.

Hawest K12 from 200 ml aerated Y2 .1% Lac, wash, into 10 ml H<sub>2</sub>O  
 Galactosidase assay: .02 ml / 10. Rel. Act. ca 1.3  
 Di      <sup>20 ml</sup> Donp<sub>g</sub> original suspension has  
 132      309      ca 800 u/ml lactase.

K12 / Glucose from 5/11/50. used for assay

For manometric assay, dilute this suspension 1/1+2, to a final  
 concn. buffer of 7/20. Cell densities (.1/10) dilute to 1/1+1 likewise

<sup>L see page 110</sup> Lactic acid should have  
 ca 250 units of activity/ml. 1 cm. flask  
 contains at 1 ml

Flask	Side 1	Side 2	4 <sup>25</sup>	4 <sup>32</sup>	4 <sup>35</sup>	4 <sup>40</sup>	4 <sup>45</sup>	5 <sup>00</sup>
A 1 G	glucose		39'	40 <sup>39</sup> ↓	42'	48	49'	
A 2 G	lactose		31	30	32'	33'	33	
A 3 G	lactose .2ml L		43	42	43	47	46	
A 4 G	lactose .1ml 74% Dextr.		55	55	58	58	58	
A 5 L	glucose		43	43	53	108	159	
A 6 L	lactose		46	45	48	83	117	
A 7 G	-		63	64	66	62	65	
A 8 L	-		42	44	45	40	45	
A 9 -	-		133	135	133	129	133	

6 mg n-glycose

N.G.